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FOREWORD

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1. INTRODUCTION

The goal of this program was to bring the PI up to speed in the field of prostate cancer research sufficiently well to allow him to write credible proposals and otherwise join the field of researchers in that field. The PI has had a long and celebrated career in optics, signal processing, pattern recognition, and the like; so naturally he sought to combine his new interests with his old skills. Assisting him in this was Dr. James Eliason of the Barbara Ann Karmanos Cancer Institute in Detroit who served as his mentor. The goals were met quite well in our judgement. We have gathered all of the conceptual parts needed to propose a totally new and extremely promising blood test for prostate (and other) cancer. It will be a test superior to PSA screening in two useful respects:

- It will not feel embarrassing or invasive (a major boon among some minority men) and
- It will have fewer false positives (saving both money and anxiety).

The same test should work to allow us to detect hard-to-spot cancers early enough to have successful interventions and to allow the physicians to track the progress of long term treatments. The details are still being worked out, but we expect to be able to submit one or more strong proposals in this area within CY 1999.

What follows is a summary of some of the things learned during this study. Here is a list of the key areas:

- Prostate cancer in the African-American population.
- Blood tests for cancer.
- Underlying hardware for cell scanning.
- IR Raman spectroscopic studies at Fisk University.
- Need for ultrafast analysis and potential means to solve the problem.
- False positive and false negative tradeoff problem and a potential solution.

Clearly, this understanding of the problems and the potential solutions points toward a system that may solve the problems and create the blood test referred to above. We are continuing the system integration studies on our own preparatory to writing a proposal on the system.

2. PROSTATE CANCER IN THE AFRICAN-AMERICAN POPULATION

Prostate cancer is the most commonly diagnosed non-skin cancer in America. The mortality rate of prostate carcinoma between the ages of 40 and 60 years is nearly 3-fold greater in the African-American population than in the white American population and at each stage of progression it appears to have a more aggressive phenotype [1]. This argues that early testing would be valuable for this population. It would clearly be beneficial to have a convenient, non-embarrassing test to detect the presence of the disease, so that the potential for cure would be increased. The ideal screening test for cancer must be sensitive so that tumors are not missed, specific to avoid the anxiety and possibility of needless surgery associated with a false diagnosis, and inexpensive so that it is cost effective for mass screening.

3. BLOOD TEST FOR PROSTATE CANCER CELLS

The current, widely used blood test for prostate cancer measures prostate specific antigen (PSA). However, this is unreliable for wide spread screening to diagnose prostate cancer. This is because normal tissues also express PSA. In fact, PSA has been shown to be present in a subpopulation of peripheral blood cells [2]. Recent studies have shown that tumor cells can be found in the peripheral blood of nearly all patients with known tumors. These cells are shed from the tumor and because most tumors of epithelial origin, they can be readily differentiate from mesothelial derived bone marrow cells on the basis of expression of epithelial specific proteins like the cytokeratins [3]. These can be thought of as tissue markers rather than tumor markers and thus are more stably expressed by the cancer cells. Relatively few normal epithelial are detected in blood and bone marrow samples, so the shedding phenomenon does appear to be cancer specific. Definitive diagnosis of cancer does require the expertise of a trained pathologist, which is the current “gold standard”.

Several methods are available for detecting cytokeratins in cells present in blood. The best specificity appears to be using immunocytochemistry with specific antibodies [4]. We estimate that the required sensitivity for a technique to screen blood samples would be to detect 1 tumor in 10^8 blood cells. The detection limits of the current assays are on the order of 1 tumor cell in 10^4 for flow cytometric methods to 1 in 10^6 for

polymerase chain reaction (PCR) and immunocytochemical assays. The PCR assays are sensitive and rapid, but lack specificity, particularly with peripheral blood samples [5, 6]. The immunocytochemical assays are time consuming and subjective.

Adding a pre-enrichment step for the cancer cells can increase the sensitivities of the various assays. The technique used most frequently for this purpose is immunomagnetic separation. Several types of immunomagnetic beads are commercially available that can be used. Enrichments up to 100-fold can be achieved with excellent recoveries (50-80%) [2, 3]. These techniques require large quantities of antibodies making them quite costly.

4. UNDERLYING HARDWARE FOR CELL SCANNING

The objective is to scan a laser beam across many cells very quickly or, conversely, to scan many cells under a laser beam very quickly. These two ways to solve the scanning problem are called scanning cytology and flow cytology, respectively. Both are well known to readers of this report, so their principles need not be reviewed. We have tentatively opted for the scanning cytometer approach, because it allows the automated system to flag the rare suspect cells for technician or physician verification. Thus the automatic identification system can err on the side of high false positives while still leaving a human a simple task of eliminating the few false positives so flagged. The human simply looks at cell after cell automatically presented to him for verification. This is a good mix of human and machine skills.

5. IR RAMAN SPECTROSCOPIC STUDIES AT FISK UNIVERSITY

Raman spectroscopy is widely recognized as the best available means to recognize molecular structures, bacteria, viruses, etc. by their spectral properties. Unfortunately, Raman spectroscopy suffers from several profound drawbacks. First, it is a very weak effect. Second, the strength of the signal decreases as the fourth power of the stimulating wavelength. This means we would like to operate in the ultra violet. But ultraviolet and even visible stimulating light causes far too much fluorescence which would swamp the Raman scattered light. Accordingly, we must work in the infrared where the effect is even weaker.

The difficulty with the extreme weakness of the signal is further compounded by the fact that we must scan so many cells that the allowable integration time on any one of them is very small indeed. So Raman spectroscopy for cell ID scanning has never been seriously proposed because it is a very weak effect which must be produced at a very unfavorable wavelength and integrated for a very short time. Without some drastic changes somewhere in the system, this is a sure recipe for failure.

Obviously, we believe we have found several means to make it work despite those problems. The system studies are now going on to be sure before we submit a proposal. One part of the solution is Surface Enhanced Raman- a method moderately well known in spectroscopy which can enhance the Raman effect by a factor of a million or more. The other part is the spectral correlator that is currently being designed.

We ran some preliminary laboratory tests at Fisk University to observe the reflectance spectra of normal bone marrow cells and cancer cells. The near-infrared reflectance spectra were measured at room temperatures using a Bomem Michelson Series model MB102 over a spectral range $0\text{-}15,800\text{cm}^{-1}$ (near infrared region). The method used was specular reflectance. The spectrum was recorded using a High speed Deuterated triglycine sulfate detector (DTGS). Each sample was scanned a 100 times to reduce the signal to noise ratio. The spectral resolution of the data is 4cm^{-1} . Figure 1 shows the spectra of studied samples. The samples with prostate cell lines are LNCaP, PC3/Pi3LN, and DU145. The normal bone marrow cell samples are labeled nbm. We observed from our data that there are significant between the cells of the normal bone marrow and those of the cancer cell lines. We expect more distinct distinguishing spectral features with the planned IR Raman spectroscopy that will be equipped with the high sensitive spectral correlator.

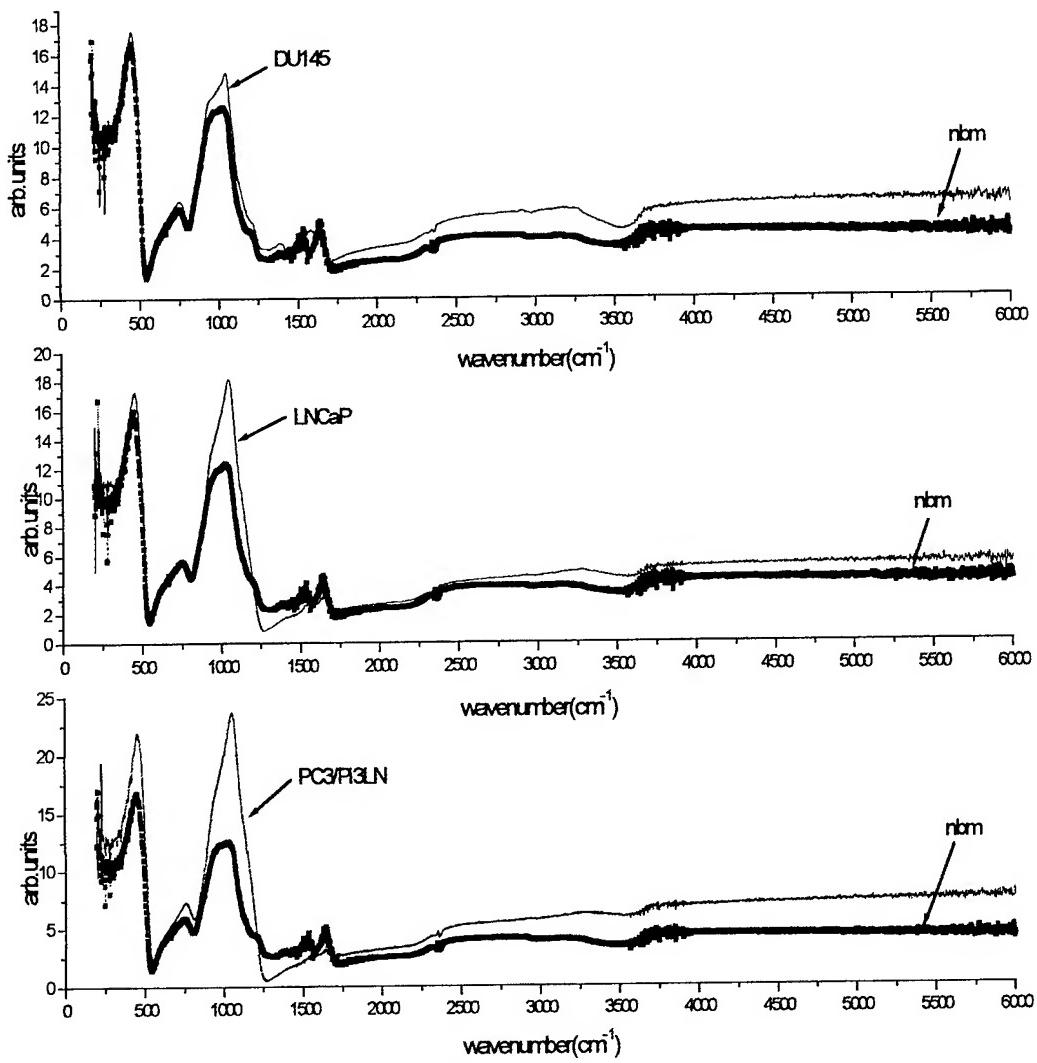


Figure 1: Near-infrared reflectance spectra of normal bone marrow cells (sample nbm) and prostate cells (samples LNCaP, PC3/Pi3LN, and DU145).

6. NEED FOR ULTRAFAST ANALYSIS AND POTENTIAL MEANS TO SOLVE THE PROBLEM

If we suppose that the event we seek has a p probability of occurring, then how many events must we sample in order to assert with some reasonable probability that such an event did not occur? The samples can be assumed to be independent, so the probability of seeing at least one event after N samples is given by the familiar binomial distribution function. The expected number is

$$\langle n \rangle = Np. \quad (1)$$

The variance is

$$\sigma^2 = Np(1-p). \quad (2)$$

We might choose to seek

$$\sigma = \langle n \rangle / 3. \quad (3)$$

That requires

$$N = 3(1-p)/p. \quad (4)$$

For p one in a million, this requires about 3 million samples. Whatever we do, we will need a great many samples. Suppose we sample only 3 million cells but want to do so in 60 seconds. That leaves us with only 20 microseconds to step from one cell to the next and take whatever spectral data on that cell we need.

The situation is even worse than that. The cells are not evenly spaced. We have a choice of slowing down to center a cell on the beam (or conversely) or of over-sampling enough to be certain to see each cell. Neither of those is desirable, but one of them must be done.

Physically scanning a slide at the required speed seems to be far too ambitious. So we imagine scanning the optical beam. Scanning speeds as high as we wish (in terms of resolvable spots per second) are available. But can we read spectra and analyze them at that rate? Not unless we somehow combine the reading and analyzing tasks in such a way that we directly read the probability of the cell's being a cancer cell on a single high quality detector. We believe that we can design such a system and that it will also enhance the reading sensitivity in two ways: allowing one super sensitive detector to be

used at all times and (automatically) the multiplex advantage long touted for interferometric spectroscopy.

7. FALSE POSITIVE AND FALSE NEGATIVE TRADEOFF PROBLEM AND A POTENTIAL SOLUTION

Part of the solution to this critical problem has already been given. In the automatic prescreening stage, we will deliberately err on the side of allowing false positives to drive our false negative rate acceptably low. Then, we will use humans to screen only the prescreened cells to reduce the false positive rate as low as possible. But we have also been working with a new, unpublished way of analyzing the data which has the property that it will classify all members of the training set perfectly even on the little data we will take (one carefully chosen Raman spectral correlation per cell). This will have the effect of making the false positive rate much smaller than if we analyzed those same data in any other way.

8. CONCLUSION

This report covered concepts, devices and experiments performed at Fisk University (an HBCU) by its PI and his collaborators there with the help of his medical mentor at Karmanos Cancer Institute. They also enlisted specialized help from two small R&D companies in order to perform their system study and design thoroughly and efficiently.

The goals of the work were achieved. This project has allowed an HBCU to become involved in the solution to a major problem which disproportionately affects African Americans-prostate cancer. We are preparing a proposal to build and validate a specialized instrument based on the work reported here. We believe that it is an exciting new set of concepts combined into a totally new system with widespread usefulness.

What has emerged from this small contract is a totally new and very promising new approach to the screening for and monitoring of cancers, especially those cancers of internal organs which are often undiagnosed until too late for effective treatment. The system will involve a scanning IR Raman spectral correlator tuned to recognize epithelial cells in blood with high discrimination and high sensitivity.

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